

ART 3A AMNT

WHAT IS CLAIMED IS:

1. A method of preparing a reconstructed non-human oocyte by transferring cell or nucleus from germinal or somatic cells into an enucleated host oocyte, which comprises the steps of:
 - a) activating said host oocyte;
 - b) enucleating said activated host oocyte when said activated oocyte is undergoing the expulsion of a second polarbody or when said activated oocyte has expelled said second polarbody (Tel-II); and
 - c) transferring nucleus from germinal or somatic cells into said enucleated host oocyte of step b) to obtain a reconstructed oocyte.
2. The method according to claim 1, wherein said transferred cell or nucleus is at nuclear stage G0, G1, S, G2, or M.
3. The method of claim 1, wherein said germinal or somatic cells of step c) are cultured prior to nucleus transfer.
4. The method of claim 1, wherein said oocyte of step a) is a secondary oocyte (M-II) and said activation is performed by artificial means selected from the group consisting of physical means and chemical means.
5. The method of claim 4, wherein said chemical means is ethanol or ionomycin.
6. The method of claim 4, wherein said physical means is selected from the group consisting of

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electrical means, thermal means, and irradiation technology.

7. The method of claim 1, wherein step b) is performed after oocytes are cultured for a period of time sufficient to allow for at least partial extrusion of a second polarbody.

8. The method of claim 1, wherein step b) is performed with oocytes in a medium with cytoskeletal inhibitors.

9. The method of claim 7, wherein step b) is effected by microsurgically removing said second polarbody with a portion of the cytoplasm containing chromosomes surrounding said at least partially extruded second polarbody.

10. A method of reconstituting a non-human embryo, which comprises the steps of:

- a) activating oocyte by artificial or natural means;
- b) enucleating said activated oocyte when said activated oocyte is undergoing the expulsion of a second polarbody or when said activated oocyte has recently expelled second polarbody (Tel-II);
- c) culturing germlinal or somatic cell prior to nucleus transfer;
- d) transferring a nucleus from said cell of step c) in said enucleated oocyte to obtain a reconstructed oocyte with a diploid chromosomal content; and
- e) culturing *in vitro* said reconstructed oocyte and/or transferring said reconstructed

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oocyte into a reproductive tract of a suitable surrogate mother to enable development into a non-human embryo.

11. The method according to claim 10, wherein said transferred cell or nucleus is at nuclear stage G0, G1, S, G2, or M.

12. The method of claim 10, wherein said oocyte of step a) is a secondary oocyte (M-II) and said artificial means is physical or chemical means.

13. The method of claim 12, wherein said chemical means is ethanol or ionomycin.

14. The method of claim 12, wherein said physical means is selected from the group consisting of electrical means, thermal means, and irradiation technology.

15. The method of claim 13, wherein step b) is performed after oocytes are cultured for a period of time sufficient to allow for at least partial extrusion of a second polarbody.

16. The method of claim 15, wherein step b) is performed with oocytes in a medium with cytoskeletal inhibitors.

17. The method of claim 15, wherein step b) is effected by microsurgically removing said second polarbody with a portion of the cytoplasm containing chromosomes surrounding said at least partially extruded second polarbody.

18. The method of claim 17, wherein step c) is effected by introducing a single cell containing a diploid nucleus into said enucleated oocyte by cell fusion or by microinjection.

19. The method of claim 10, wherein said non-human embryo develops into a non-human animal.

20. A method for production of a transgenic non-human embryo, which comprises the steps of:

- a) activating oocyte by artificial or natural means;
- b) enucleating said activated oocyte when said activated oocyte is undergoing the expulsion of a second polarbody or when said activated oocyte has recently expelled second polarbody (Tel-II);
- c) culturing germinal or somatic cell prior to nucleus transfer;
- d) transferring a transgenic nucleus from said cell of step c) transfected with a desired DNA construct in said enucleated oocyte to obtain a reconstructed oocyte with a diploid chromosomal content; and
- e) culturing *in vitro* said reconstructed oocyte and/or transferring said reconstructed oocyte into a reproductive tract of a suitable surrogate mother to enable development into a non-human embryo.

21. The method according to claim 20, wherein said transferred cell or nucleus is at nuclear stage G0, G1, S, G2, or M.

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22. The method according to claim 20, which further comprises developing said non-human embryo into a fetus.

23. The method according to claim 22, which further comprises developing said fetus into an offspring.

24. The method of claim 20, wherein said non-human embryo develops into a non-human animal.

25. A transgenic embryo obtained according to the method which comprises the steps of:

- a) activating oocyte by artificial or natural means;
- b) enucleating said activated oocyte when said activated oocyte is undergoing the expulsion of a second polarbody or when said activated oocyte has recently expelled second polarbody (Tel-II);
- c) culturing germlinal or somatic cell prior to nucleus transfer;
- d) transferring a transgenic nucleus from said cell of step c) transfected with a desired DNA construct in said enucleated oocyte to obtain a reconstructed oocyte with a diploid chromosomal content; and
- e) culturing *in vitro* said reconstructed oocyte and/or transferring said reconstructed oocyte into a reproductive tract of a suitable surrogate mother to enable development into a non-human embryo.

26. A transgenic fetus obtained according to the method which comprises the steps of:

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- a) activating oocyte by artificial or natural means;
- b) enucleating said activated oocyte when said activated oocyte is undergoing the expulsion of a second polarbody or when said activated oocyte has recently expelled second polarbody (Tel-II);
- c) culturing germinal or somatic cell prior to nucleus transfer;
- d) transferring a transgenic nucleus from said cell of step c) transfected with a desired DNA construct in said enucleated oocyte to obtain a reconstructed oocyte with a diploid chromosomal content; and
- e) culturing *in vitro* said reconstructed oocyte and/or transferring said reconstructed oocyte into a reproductive tract of a suitable surrogate mother to enable development into a non-human embryo.

27. A transgenic offspring obtained according to the method which comprises the steps of:

- a) activating oocyte by artificial or natural means;
- b) enucleating said activated oocyte when said activated oocyte is undergoing the expulsion of a second polarbody or when said activated oocyte has recently expelled second polarbody (Tel-II);
- c) culturing germinal or somatic cell prior to nucleus transfer;
- d) transferring a transgenic nucleus from said cell of step c) transfected with a desired DNA construct in said enucleated oocyte to

obtain a reconstructed oocyte with a diploid chromosomal content; and

- e) culturing *in vitro* said reconstructed oocyte and/or transferring said reconstructed oocyte into a reproductive tract of a suitable surrogate mother to enable development into a non-human embryo..

28. A method of cloning a non-human animal by cell or nuclear transfer which comprises the steps of :

- a) activating oocyte by artificial means;
- b) enucleating said activated oocyte when said activated oocyte is undergoing the expulsion of a second polarbody or when said activated oocyte has recently expelled second polarbody (Tel-II);
- c) culturing germinal or somatic cell prior to nucleus transfer;
- d) transferring a diploid nucleus from said cell of step c) in said enucleated oocyte to obtain a reconstructed oocyte with a diploid chromosomal content; and
- e) culturing *in vitro* said reconstructed oocyte and/or transferring said reconstructed oocyte into a reproductive tract of a suitable surrogate mother to enable development into a non-human embryo.

29. The method according to claim 28, wherein said transferred cell or nucleus is at nuclear stage G0, G1, S, G2, or M.

30. The method of claim 28, wherein said oocyte of step a) is a secondary oocyte (M-II) and said artificial means is physical or chemical means.

APPENDIX

31. The method of claim 30, wherein said chemical means is ethanol or ionomycin.

32. The method of claim 30, wherein said physical means is selected from the group consisting of electrical means, thermal means, and irradiation technology.

33. The method of claim 28, wherein step b) is performed after oocytes are cultured for a period of time sufficient to allow for at least partial extrusion of a second polarbody.

34. The method of claim 30, wherein step b) is performed with oocytes in a medium with cytoskeletal inhibitors.

35. The method of claim 31, wherein step b) is effected by microsurgically removing said second polarbody with a portion of the cytoplasm containing chromosomes surrounding said at least partially extruded second polarbody.

36. The method of claim 32, wherein step c) is effected by introducing a single cell containing a diploid nucleus into said enucleated oocyte by cell fusion or by microinjection.

37. The method of claim 28, wherein said nucleus or cell of step c) is transgenic or non-transgenic.

38. The method of claim 28, wherein said non-human embryo develops into a non-human animal.

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